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Preferences for nesting material as environmental enrichment for laboratory mice

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Summary

Behavioural and psychological needs of laboratory animals generally cannot adequately be met in standard laboratory cages. Environmental enrichment, which provides a more structured environment can enhance the well-being of laboratory animals. They may perform more of their species-specific behaviour and may control their environment in a better way. An easily applicable form of enrichment for laboratory mice is nesting material. Six different types of nesting materials were evaluated in a preference test with male and female animals of two strains (C57BL/6J or BALB/c, $n=48$). No significant differences in preference were found between the strains or between the sexes.

All mice showed a clear preference for cages with tissues or towels as compared to paper strips or no nesting material, and for cages with cotton string or wood-wool as compared to wood shavings or no nesting material. Paper-derived materials were preferred over wood-derived materials, although the results also suggest that the nature (paper or wood) of the nesting material is less important than its structure, which determines the nestability of the material. Nesting material may be a relatively simple method to contribute to the well-being of laboratory mice.

Keywords Preference tests; environmental enrichment; nesting material; nestbuilding; mice

Standard laboratory cages are designed to fulfil the most essential needs in a laboratory animal's life, such as provision of food, water and a substrate, e.g. bedding or a grid floor, to avoid contact with their excreta. Animals however, also have behavioural and psychological needs, most of which cannot be met in these cages. The animals are able to perform only a part of their complete species-specific behavioural repertoire (Van de Weerd *et al.* 1994), which can result in abnormal behaviour such as stereotypies (Wemelsfelder 1990). Furthermore, animals maintained in

unresponsive environments and highly unnatural groupings are less adequate models for extrapolating experimental results to humans (Markowitz & Gavazzi 1995). Environmental enrichment may enhance the well-being of laboratory animals by providing a more structured environment which enables them to perform more of their species-specific behaviour and which gives them more control over their environment (Scharmann 1991, Chamove 1989). As a consequence, animals from enriched environments may be more physiologically and psychologically stable and better representatives of the species and thus ensure better data collection and scientific results (Benn 1995, Scharmann 1991, Markowitz & Gavazzi 1995). The introduced enrichment

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should be interesting for the animals by meeting their behavioural requirements but, from the human point of view, it should be easy to provide, remove and clean (Van de Weerd & Baumans 1995).

Nesting material is an easily applicable form of enrichment for laboratory mice. Both males and females will build a nest when offered nesting materials (Lisk *et al.* 1969, Lee 1972, 1973). Females build nests during pregnancy to bear and raise their young (Lisk *et al.* 1969, Broida & Svare 1982). Hormones such as progesterone play an important role in this maternal nestbuilding (Lisk *et al.* 1969). Between strains both qualitative and quantitative differences in maternal nestbuilding exist (Broida & Svare 1982). In laboratory mice, the use of nesting material reduces the preweaning mortality of pups and enhances the number of litters (Porter & Lane-Petter 1965). Similar results have been reported for rats (Nolen & Alexander 1966). Norris and Adams (1976) found that the type of nesting material used markedly affects the preweaning survival rates in the rat.

Nesting material is also used, both by females and males, as a source of protection e.g. against extreme environmental temperatures (Lisk *et al.* 1969). Behavioural adaptations, such as nestbuilding, must be used for temperature regulation, when the physiological systems alone are inadequate to maintain body temperature (Lynch & Hegmann 1972). Lee and Wong (1970) showed that the amount of cotton used for nestbuilding increased with decreasing temperatures, although significant differences were found between strains of mice. Nests also offer an opportunity to hide from predators and in the laboratory to avoid aggressive conspecifics, or to provide a shelter from overexposure to light.

When applying enrichment, it is necessary to evaluate the suitability of the enrichment programme, as various species or strains may respond differently to the methods of enrichment (Beaver 1989). Preference tests can be used to determine some general principles about species relevant properties of enrichment devices (Mench 1994). Choice tests have been used to assess the relative preference for or avoidance of several housing

conditions in laboratory animals (Blom *et al.* 1992). Blom *et al.* (1996) and Mulder (1975) offered mice different types of bedding material. Ottoni and Ades (1991) allowed hamsters to choose between nestboxes in relation to food and nesting material and gerbils were offered a choice between partially darkened or transparent cages (Van den Broek *et al.* 1995). Pregnant mice and rats, chose wood-derived bedding over beddings with other origins (e.g. clay, corn cob) to build nests before parturition (Mulder 1974, 1975). In Blom's study (1996), mice showed a preference for shredded filter paper in comparison with smaller particled bedding material.

Nesting material has been studied mainly in relation with pregnancy or cold exposure. The aim of the present study was to investigate whether the nesting materials tested may serve as enrichment and to detect possible differences in preference for these materials between or within strains of mice. For this purpose male and non-pregnant female mice of two inbred strains were tested in a preference test and their choices for different types of nesting material (wood or paper derived) added to otherwise standard environmental conditions were evaluated.

Animals, materials and methods

Animals

Female and male mice of two strains (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU, $n=48$) were used. They were bred and raised without any nesting material. At the start of the experiment they were 8–10 weeks of age. The experiment was conducted in two cohorts, the first experiment (male mice) lasted seven weeks, the second (female mice) lasted four weeks. The animals were housed (per strain and sex) in groups of six animals in a housing system, consisting of two Macrolon type II cages (375 mm², UNO Roestvaststaal, Zevenaar, The Netherlands), connected with a passage tube, similar to the tubes used in the preference test system. Both cages were supplied with food pellets *ad libitum* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad libitum* and sawdust bedding

(Lignocel 3/4, Rettenmaier and Sohne, Ellwangen Holzmuhle, Germany). The animals were kept in conventional rooms with controlled photo period (12:12 L:D, lights on at 07:00 h, approx. 200 lux at 1 m above the floor), temperature (20–22 °C), relative humidity (50–60%) and ventilation (15 air changes per hour). Environmental conditions in the experimental rooms were similar, except for the light intensity which was approximately 300 lux at 1 m above the floor, in order to approach light intensities in standard animal rooms.

Preference test system

The preference test system used in this study has been validated and described in detail by Blom *et al.* (1992). In short, a multiple housing system was used consisting of either two or four test cages (Macrolon type II) connected by non-transparent tubes (PVC, inner dimensions: 2.6 × 2.6 × 25 cm) to a central cage (15 × 15 × 18 cm, transparent perspex). When testing with a two-cage system the central cage was divided diagonally by a PVC sheet (19 × 17 cm). A total of six multiple housing systems were used divided over two four-tiered constructions in two similar experimental rooms. Each construction was turned gently during testing to prevent bias due to external influences in the experimental room which could interfere with the choice behaviour of the mouse.

The test cages were supplied with 50 g of sawdust bedding (Lignocel 3/4), a food hopper with equal amounts of food pellets (100 g, RMH-B) and tap water in bottles. The central cage had no food, water or bedding. The movements of the mice between the test cages were detected automatically by means of photo-electrical devices in the passage tubes. The signals were sent to a computer which calculated dwelling times per cage (software: Gate-Watch, Metris System Engineering, Leiden, The Netherlands).

Behavioural observations

One of the six multiple housing systems was equipped with a video camera system. Each test cage, including the central one, was provided with a video camera (Panasonic

WV-1510). The cameras were connected with the photo-electrical devices, so that movements of the mouse could be followed in the test system. The signals from the video cameras were sent to a time-lapse video recorder (Panasonic AG-6700) which could record 24 h of testing (recording: 1/9 of normal speed). During the night the experimental room with the video equipment had red lights (approx. 5 lux at 1 m) to enable video recordings.

Procedure; nesting materials

Mice were introduced into the test system between 15:00 and 17:00 h and tested individually during 48 h. A group of six mice (of one sex and one strain) were tested simultaneously. The behaviour of one animal was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). Food and water of each test cage were weighed before and after the experiment.

Three test series were performed to test six different types of nesting material. In each of the first two series (series 1: paper, series 2: wood), three nesting materials were compared (the fourth cage in each series contained sawdust bedding without nesting material). In the third series, per strain and sex the nesting materials which most animals had chosen in the first two series were compared in a two-cage system. Figure 1 shows the nesting materials and Table 1 describes the materials and gives the amounts provided per series (approximately equal volumes).

Statistical analysis

The dwelling data were analysed by distinguishing three time frames: the total of dwelling times during the 48 h of the experiment, the dwelling times during 12 h of day light (second day of the test) and the dwelling times of 12 h of night time (second night of the test). These two latter periods synchronized the periods of collected behavioural data (video tape recordings).

The method of statistical analysis used has been described by Blom *et al.* (1995). Briefly, per test series the dwelling time data (in



Fig 1 The six different nesting materials tested in the preference test. Top row (paper series): paper towel (left), tissues (middle), folded paper strips (right). Bottom row (wood series): cotton string (left), wood-wool (middle), wood shavings (right)

Table 1 Nesting materials

Material	Trade name	Appearance (size)	Amount
Paper	Paper towel ¹	Sheet (25 × 31 cm)	1 piece
	Kleenex ^R	Sheet (20 × 21 cm)	2 pieces
	tissues ²		
	Enviro-dri ³	Folded strips (11 × 0.3 cm)	5 g
Wood	Sharpy ⁴	Cotton string (variable)	5 g
	Wood-wool ³	Strips (variable)	5 g
	Gold shavings ³	Wood shavings (variable)	5 g

¹Celtona, Cuijk, The Netherlands, ²Kimberly-Clark Corporation³, EEC, ³BMI, Helmond, The Netherlands, ⁴VNK, Haarlem, The Netherlands

seconds) were logarithmically transformed as they were not always normally distributed, and to increase the independency of the data. For the same reason, central cage dwelling times were not included in the analysis. Data on food and water intake were not transformed, because they were normally distributed.

The data were analysed using multivariate repeated measures analysis (Wilk's lambda) to evaluate the influence of type of nesting material and interactions on choice behaviour and to detect possible differences between the strains, or sexes of a strain in choice behaviour. Food and water intake were analysed in a similar way as the

dwelling times. Statistical significance was preset at $P < 0.05$.

Overall significant differences between choice cages in dwelling times and amount of food and water consumed were further analysed using a paired *t*-test to indicate which of the cages were preferred or avoided. As multiple comparisons were made, the level of statistical significance was preset at $P < 0.0083$ (Bonferroni's adaptation).

Behavioural data

The behavioural data on video tape were viewed and analysed using a behavioural observation software package (The Observer v 2.0, Noldus Info Tech B.V., The Netherlands). The tapes were viewed at normal speed, thus behaviour was seen nine times faster than the original behaviour. Every 5 s the behaviour was scored, which corresponds to one sample every 45 s in reality. The following ethogram was used to classify the behaviour (based upon Blom *et al.* 1992):

Sleeping (sl)=

movements are absent while the animal is in a sitting or lying position. Very short or minor movements during a long resting period (e.g. turning) are not considered as an interruption.

Manipulation (man)=

manipulation of the nesting material, includes shredding, fraying, dragging and nestbuilding behaviour.

Grooming in the nest (gr-i)=

while sitting or standing in its nest, the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

Grooming outside the nest (gr-o)=

same as gr-i, but outside the nest.

Ingestive behaviour (ing)=

includes eating and drinking behaviour. Eating: gnawing on food particles from the food hopper or from the sawdust, coprophagy is included as well. Drinking: licking the nipple of the drinking bottle.

Locomotion (loco)=

all other movements (e.g. walking, running, jumping).

Climbing (cli)=

climbing on or hanging from the bars of the wire cage lid or food hopper, or

standing on the passage tube or drinking nipple. While climbing or hanging the hind legs or tail may touch the cage walls.

Rearing (rear)=

standing position with the forepaws not touching the cage floor. The animal is standing on its hind feet or toes, usually supporting itself with the tail. The fore-paws may lean against the passage tube, cage wall or food hopper.

Digging (dig)=

bedding material is pushed forwards or backwards with nose, fore paws or hind legs. Mouse moves around or is sitting in one place.

Descriptive statistics were used to analyse the behavioural data, because only two animals from each sex and strain group ($n=12$) were observed per test series. The results were used to describe the behaviour of the mice in the different test cages during a test series. The distribution of behaviour in each test cage was analysed for the night and day time period separately.

Results

Figure 2 illustrates the mean relative dwelling times per cage for the paper series. Figures 3 and 4 show the same for the wood series and the paper vs wood series, respectively. Figure 5 gives two examples of the distribution of behaviour for the four cages in the paper and wood series.

Cage choice

No significant differences in cage preferences (dwelling times) were found between strains in the paper series and the wood series, except for the total data of the paper series (MANOVA, $P<0.05$). The cause for this difference is not very clear. Also, no significant differences between the sexes of a strain were found, in all three test series and during all three time periods. Therefore, per strain both sexes were analysed together in the paired *t*-test analysis. Mice of both strains chose significantly for a cage during all three time periods of both the paper and wood series (MANOVA, for all: $P<0.001$). Figure 2 illustrates that in the paper series the cages

with the tissues and paper towels were preferred, whereas from Fig. 3 it can be concluded that in the wood series the cages with the cotton string and wood-wool were preferred. In the paper series the biggest contrasts between cages were found to exist between the cages with tissues and towel on the one hand and the cage with paper strips and the cage without nesting material on the other. This was most clear for mice of the BALB/c strain (paired *t*-test, all $P<0.001$). For the C57BL mice it was less obvious. The differences between the cages with either tissues or towel and the cage without nesting material were significant (paired *t*-test, all $P<0.01$), just as the contrast between the cages with the towel and paper strips (paired *t*-test, all $P<0.005$).

In the wood series mice of both strains spent significantly more time in the cages with cotton string and wood-wool in comparison to the cage with wood shavings and the cage without nesting material (both strains: paired *t*-test, all $P<0.005$). Only during night periods the contrast between the cage with the wood-wool and the cages with wood shavings or without nesting material were not significant for the C57BL. For the C57BL strain significant differences between the cages with the cotton string and wood-wool were found (paired *t*-test, all $P<0.005$).

In the paper vs wood series (Fig. 4) the materials which most mice had chosen in the paper and in the wood series were compared, being the paper towels vs cotton string for the C57BL males, and the tissues vs wood-wool for the BALB/c males. Females of both strains preferred the tissues and cotton string. Data were analysed per strain. The C57BL mice chose significantly for the cage with the paper nesting material (males: towels, females: tissues), but only during the total and day period (MANOVA, both: $P<0.005$). BALB/c mice did not make significant cage choices during all three time periods of this series.

Food and water intake

Significant differences between the strains in food and water intake were found in the wood series only (MANOVA, both: $P<0.05$).

NESTING MATERIAL: PAPER SERIES

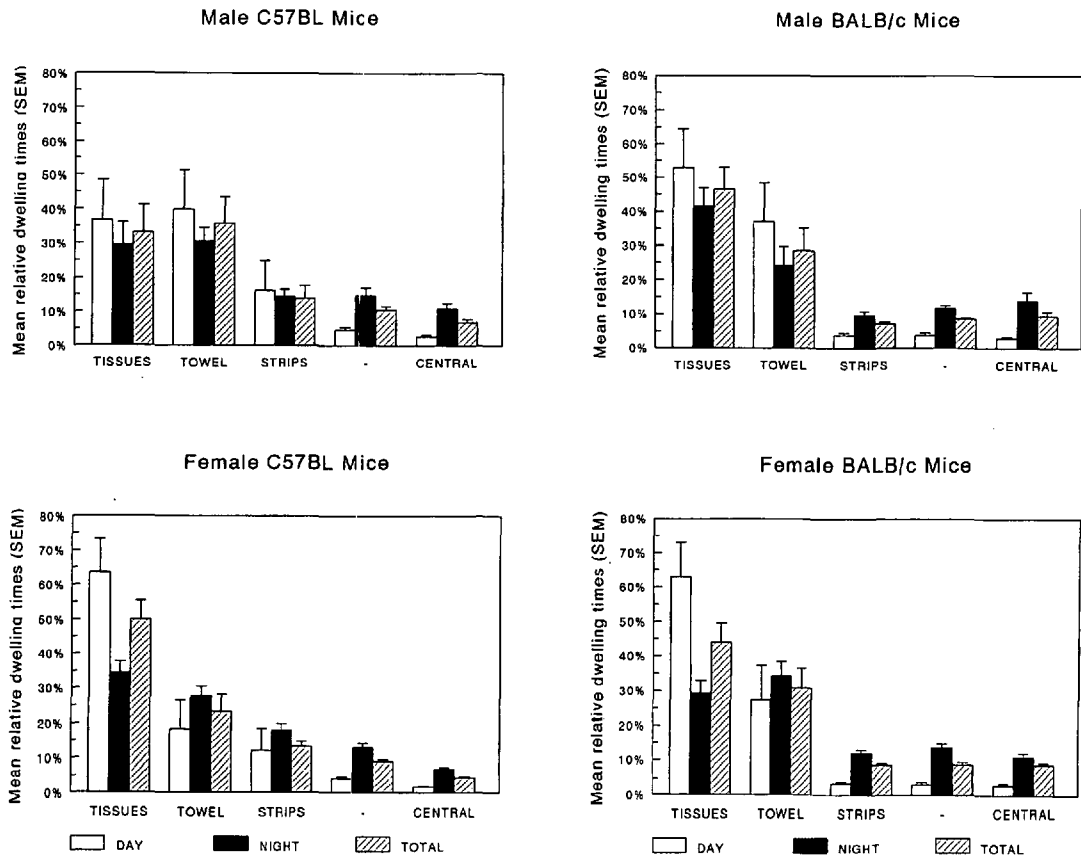


Fig 2 Results of the preference test with three types of paper-derived nesting material. Mean relative dwelling times (and SEM) per cage for day (=12 h), night (=12 h) and total (=48 h) period, for mice of two strains ($n=48$)

BALB/c mice consumed overall more food than the C57BL mice. In the paper and wood series no significant differences between the sexes of the strains were found in cage choice for food or water intake.

In the paper series mice of both strains made significant cage choices for water consumption, but not for food consumption. C57BL mice drank most in the cages with tissues and no nesting material (MANOVA, $P<0.001$); BALB/c mice in the cages with tissues and towels (MANOVA, $P<0.005$). Significant contrasts were found between the cage with paper strips and the cage without nesting material (C57BL: paired t -test, $P<0.01$), and the cages with paper strips and tissues (paired t -test, C57BL: $P<0.01$; BALB/

c: $P<0.001$). In the wood series significant cage choices for food intake were made by the BALB/c mice, they ate most in the cages with wood shavings and no nesting material (MANOVA, $P<0.005$). The main contrasts were found between the cages with wood shavings and either wood-wool or cotton string (BALB/c: paired t -test, $P<0.01$). Significant cage choices for water consumption were made by the C57BL mice, they drank most in the cages with cotton string and no nesting material (MANOVA, $P<0.05$).

In the paper vs wood series, food and water intake of the BALB/c mice did not differ significantly between the cages. The C57BL mice did not make a significant cage choice for water consumption, but the male mice

ate significantly more in the cage with the paper towel than in the other cage (MANOVA, $P < 0.05$), whereas the females did not make a significant cage choice.

Behavioural data

A striking behaviour performed by approximately half the number of animals, is the combining of nesting materials by dragging material from one cage to another. In the paper series the animals that dragged, always made a combination of tissues and towel. In the wood series the cotton string was combined with wood-wool and sometimes a few wood shavings were added. In general, the preferred materials were combined.

Figure 5 illustrates the distribution of

behaviour during daytime and during the night for the BALB/c mice (paper series) and the C57BL mice (wood series). During daytime the mice mostly slept in their preferred cage, where they also showed manipulation of the nesting material and grooming in the nest. During the night the mice were more active and they performed active behaviours (ingestive behaviour, locomotion, climbing, rearing, digging) in all cages of the test system, although a fair amount of sleeping was performed in the preferred cage.

Discussion

The results indicate that mice preferred cages with nesting material and that they discriminate between different nesting materials

NESTING MATERIAL: WOOD SERIES

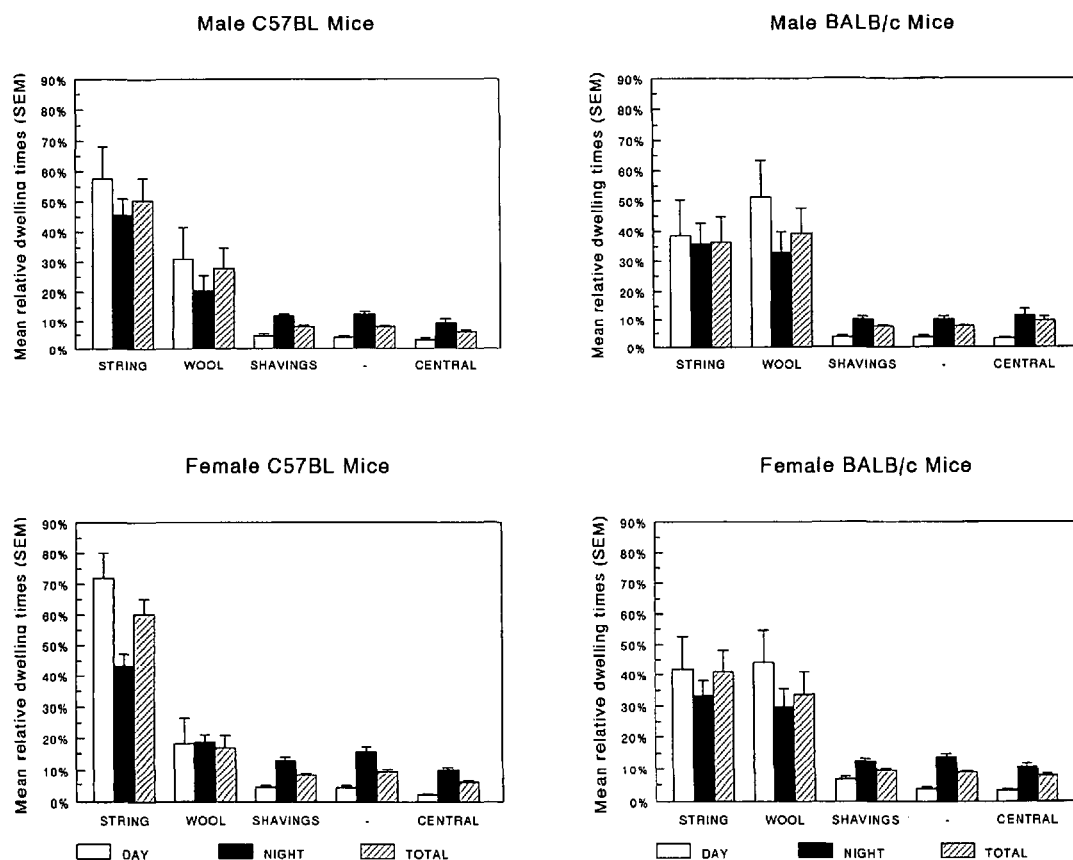


Fig 3 Results of the preference test with three types of wood-derived nesting material. Mean relative dwelling times (and SEM) per cage for day (=12 h), night (=12 h) and total (=48 h) period, for mice of two strains (n=48)

NESTING MATERIAL: PAPER vs WOOD

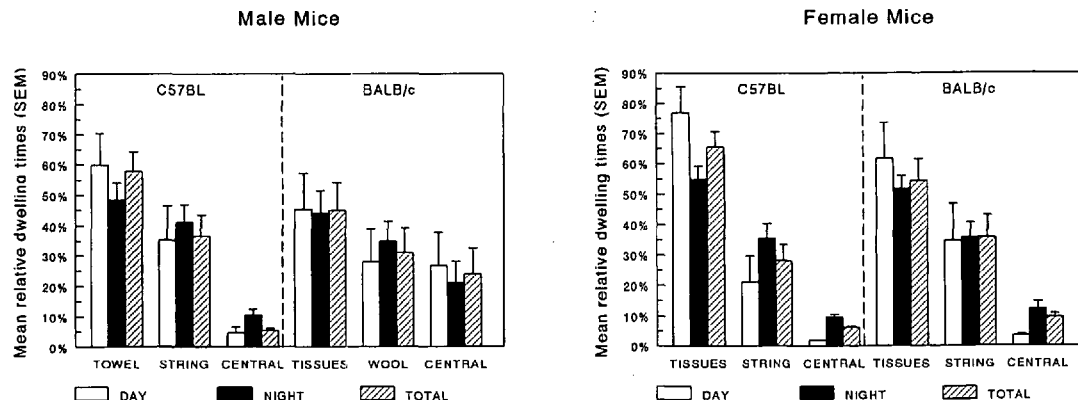


Fig 4 Results of the preference test with the two most preferred nesting materials from the paper and wood series (as indicated). Mean relative dwelling times (and SEM) per cage for day (=12 h), night (=12 h) and total (=48 h) period, for mice of two strains (n=48)

BEHAVIOUR

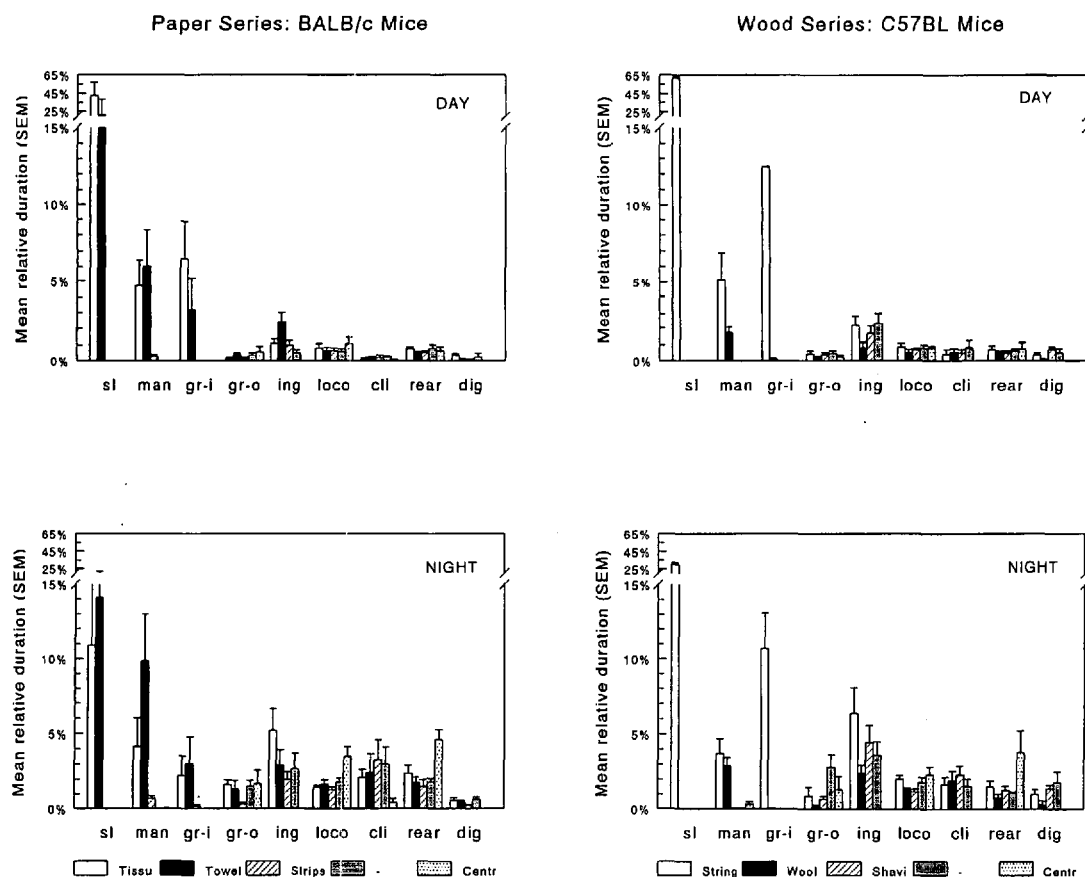


Fig 5 Results of behavioural observations from preference tests for nesting material with mice of two strains (n=8). Mean relative time (and SEM) spent on indicated behaviour during day (=12 h) and night (=12 h) period (see Animals, Materials and Methods for explanation of abbreviations)

and make consistent choices when submitted to a preference test. Cages with tissues or towels are preferred over cages with paper strips, whereas cages with cotton string or wood-wool are preferred over wood shavings as nesting material. C57BL mice preferred paper-derived nesting materials over wood-derived materials. BALB/c mice did not make a significant choice between the two materials offered, but in most cases combined them. In the third series three male BALB/c spent most time in the central cage, where they combined all nesting materials. An explanation for this behaviour might be that when testing with only two cages, a piece of PVC is dividing the central cage in two parts, making this area smaller and relatively dark.

The fact that some animals combine nesting material might suggest that there is not a clear preference for the nature of nesting material (e.g. paper or wood) but that other features of the nesting material such as the structure (e.g. shredded or as a sheet), also play a role. In a choice test with mice, Mulder (1975) found a significant preference for bedding materials from a wood origin (aspen and cedar), but he did not test paper products. Both cellulose wadding and shredded paper as well as wood chips yielded low to normal preweaning mortality in mouse litters in the study by Porter and Lane-Petter (1965). In the study by Blom *et al.* (1996), both C57BL and BALB/c mice showed a preference for shredded paper bedding instead of sawdust or wood chips. Behavioural observations indicated that manipulation of the bedding and resting in nests were performed mostly on this type of bedding. In the study by Nolen and Alexander (1996) best breeding results were found when providing the rats with shredded paper as nesting material, whereas in the study by Norris and Adams (1976) better breeding results were obtained with wood-wool instead of paper tissues. These results also suggest that the nature of the nesting material might be less important than the structure (e.g. shredded or as a sheet). The structure may be important because it determines the nestability of the material. In the present study, the characteristic feature

which the preferred nesting materials have in common is that the mice can manipulate them to build nests, and by doing this, they are able to structure their environment. Towel and tissues were shredded to build nests and the wood-wool and cotton string were shaped into the desired form. When nesting material is put into the home cages of mice they start building nests within minutes after introduction (Watson 1993, Schneider & Chenoweth 1970). With cage cleaning, these nests can be transported completely to the clean cage. In the present study several animals, especially of the BALB/c strain also combined the two preferred nesting materials to make more complicated nests. Pennycuik (1973) also observed that mice moved nesting material (wood-wool) to the nestbox selected as nest site. The behavioural observations in the present study showed that 10–20% of the time budget was spent on manipulation of the nesting material during day or night.

Another aspect of the nesting material which could be an important criterion for selection by the mice is the degree of light absorption. Mice are nocturnal animals who often prefer to hide and sleep at dark places during daytime. Exposure to light can cause damage to the eyes (Clough 1987). However, most of the nesting materials preferred in the present study allowed some penetration of light (e.g. the tissues and cotton string). Only the paper towels could provide a shelter for light, but only if the mice were completely covered by the materials of their nest, which was mostly not the case.

In general, there were no differences in cage choice between the day and night periods. During darkness the mice spent most of their time in the same cage as during light periods, which was illustrated by the behavioural observations. However, during the night period the mice also visited the other cages of the test system. In general, the results showed that the mice visited all the cages of the housing system with approximately the same frequency, however the preference is based on the duration of their stay. Since sleeping requires most time, the cage selected for resting is the preferred cage by definition (Blom *et al.* 1992). Figure 5

illustrates the increased activity of the mice during the night. In contrast with the present results, experiments with rats showed that different cages are preferred during day and night, suggesting that various behavioural activities require different cage floor covering (Van de Weerd *et al.* 1996).

The study by Blom *et al.* (1992) showed that from the recorded behaviour, 65% of the time is spent on sleeping, grooming and digging behaviour. In the present study, the same amount of time is spent on sleeping, grooming in the nest and manipulation of the nesting material. In the study by Blom *et al.* mice did not have nesting material, so it seemed that they performed digging as a kind of redirected behaviour for nestbuilding activities. In the present study nestbuilding behaviour could be performed with the nesting material and digging was less frequently observed.

Mice of both strains consumed equal amounts of food in all four cages of the paper series. However, most water was consumed in the cage provided with tissues. This was also the preferred cage for all mice, except male C57BL. In the wood series the BALB/c mice ate most in the cage without nesting material (males) and the cage with the wood shavings (females) and the C57BL mice drank mostly in the cage with the cotton string. In the third series only male C57BL mice had a preference for the cage with the paper towel to consume food. These results are not very consistent and are only partly in concordance with the results of Blom *et al.* (1996), who found that eating and drinking behaviour of the mice was similar for the four test cages with different types of floor covering. Hamsters, on the contrary, consumed most food close to their nests, because they preferred nestboxes nearest to the food source (Ottoni & Ades 1991). Food intake differed significantly between the strains, with BALB/c mice eating more than C57BL mice. This can be explained by a difference in weight; BALB/c mice are in general heavier than C57BL mice. Water intake did not differ between the strains. Although no differences between the strains were observed regarding the choice of nesting material, there was strain-specific behaviour towards the nesting material.

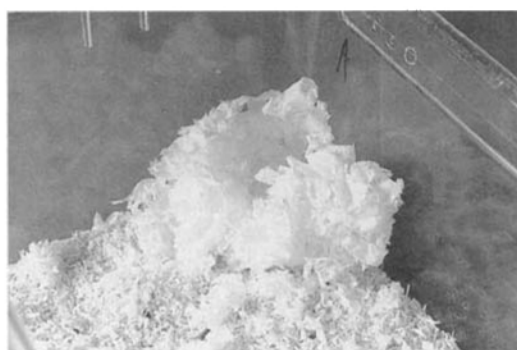


Fig 6 Dome-shaped nest made of shredded tissues by a male BALB/c mouse

Tissues and towels were shredded more thoroughly by BALB/c mice than by C57BL mice. Also the shape of the nests differed. The BALB/c mice build dome-shaped nests (Fig. 6), whereas the C57BL mice made bowl-shaped nests. This has also been described by Lee and Wong (1970). Other studies have also reported differences in the shapes of sleeping nests between mice of different strains [Lee 1972, 1973, Lynch & Hegmann 1972, 1973]. These differences in nestbuilding between strains are probably genetically determined (Lynch & Hegmann 1972).

The results of this study show that laboratory mice prefer nesting material which they can use for nestbuilding. By providing them with nesting material the animals are able to use an active strategy to manipulate and control more aspects of their environment, which is important for the effectiveness of enrichment (Mench 1994, Sluyter *et al.* 1995). Nesting material is easily applicable in standard cages and thus may be a relatively simple method to contribute to the well-being of laboratory mice.

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